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Research report

Distribution of tumor necrosis factor receptor messenger RNA in normal and herpes simplex virus infected trigeminal ganglia in the mouse

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Abstract

Purpose: to investigate the distribution of p55 and p75 tumor necrosis factor (TNF) receptor mRNA in normal murine trigeminal ganglia, and in murine trigeminal ganglia acutely infected with McKrae strain herpes simplex virus (HSV). **Methods:** in situ hybridization with antisense ³⁵S-labeled riboprobes for mRNA encoding both the p55 and p75 TNF receptor (TNFR) subtypes was used in normal and HSV-infected murine trigeminal ganglia. Sense riboprobes were used as controls. **Results:** in situ hybridization with both p55 and p75 riboprobes produced a strong autoradiographic signal over many, but not all, trigeminal sensory neurons. Signal for mRNA encoding both TNFR subtypes was also present over the arachnoid layers surrounding trigeminal ganglia. Acute ocular HSV infection was accompanied by an intense leukocytic infiltrate into the ophthalmic portion of the trigeminal ganglia, and, in this setting, increased p55 and p75 mRNA signal was closely related to the location and number of infiltrating white blood cells. The distribution and number of trigeminal sensory neurons expressing mRNA for the two TNFR subtypes did not appear to change following infection. Signal over control sections hybridized with sense p55 and p75 TNFR cRNA probes was comparable to background. **Conclusions:** the observed distribution of p55 and p75 TNFR mRNA over trigeminal sensory neurons and over the arachnoid layers surrounding trigeminal ganglia supports suggestions that TNF has a direct effect on neurons, either as a neuromodulator or neurotrophic factor, and that TNF may play a central role in blood–brain barrier regulation. Increased signal for TNFR mRNA in acutely infected trigeminal ganglia appears to reflect infiltration by receptor-bearing white blood cells.

Keywords: Cytokine; Trigeminal ganglion; Meninges; Receptor; Tumor necrosis factor (TNF)

1. Introduction

Ocular inoculation with herpes simplex virus (HSV) results in both productive and latent pathways of viral gene expression in trigeminal sensory neurons [5,23,27,29,33,34,52]. Determinants of HSV latency appear complex, and include both host and viral factors [18,48]. Much recent attention has been given to the role of the host immune system and cytokines in viral latency and reactivation [1,19,39], particularly tumor necrosis factor (TNF), a potent pro-inflammatory and mitogenic signalling molecule [9,57].

The role of TNF in HSV infection has been controver-

sial. Rossol-Voth and colleagues have shown that systemically administered TNF protects against HSV-induced mortality in vivo [42], and both Feduchi and associates [17] and Chen and colleagues [47] have described inhibition of HSV replication by TNF in cell culture. Of note, the in vitro study by Chen and associates demonstrated TNF-induced inhibition of HSV replication in both corneal fibroblasts and corneal epithelial cells [47], findings highlighted by the report of Ghiasi and associates correlating local TNF expression with decreased HSV-induced corneal scarring in mice [21]. In contrast, Waley and associates have recently reported enhanced HSV reactivation and replication in explanted trigeminal ganglia superfused with TNF [59]. Taken together, these findings suggest that the effect of TNF on HSV replication may be site and tissue dependent, determined presumably by cell-specific expression of TNF receptor (TNFR) subtypes.

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Two high affinity TNFR subtypes have been identified to date, termed p55 and p75 [9,24,57]. While trigeminal sensory neurons appear to be an important site of HSV latency, and TNF does appear to influence HSV reactivation in trigeminal ganglia [59], evidence has yet to be provided for synthesis of TNFRs by trigeminal ganglion neurons and/or neuroglial cells. We, therefore, applied a sensitive and specific *in situ* hybridization protocol [24] to examine the distribution of mRNA encoding the two TNFR subtypes in normal murine trigeminal ganglia, and in murine trigeminal ganglia acutely infected with HSV.

2. Materials and methods

Studies in this report were carried out in accordance with the Use of Laboratory Animals as adopted by the National Institutes of Health. All experiments were performed on 8- to 10-week-old female BALB/c mice (Simonsen, Gilroy, CA).

2.1. Ocular HSV infection

Mice were anesthetized with CO₂. A few drops of 0.5% proparacaine (Alcon, Fort Worth, TX) were then applied to the corneas, the epithelium was lightly scored with a sterile 27-gauge syringe, and 20–30 μ l of McKrae strain HSV (10⁹ PFU/ml) were applied topically. The mice were allowed to recover from anesthesia and maintained for 3 days following inoculation.

2.2. Tissue preparation

Deeply anesthetized normal and infected mice were perfused transcardially with 10 ml of 0.15 M NaCl, followed immediately by 20 ml of 10% neutral buffered formalin. The trigeminal ganglia were removed and allowed to postfix in the final perfusate at 4°C for 2–3 weeks, after which time they were transferred to the same perfusate with 10% sucrose added as a cryoprotectant for 12–24 h. Four 1-in-4 series of 10 μ m-thick cryostat sections through the entire trigeminal ganglia were collected onto uncoated, positively charged slides (Fisher).

2.3. *In situ* hybridization histochemistry

Bluescript SK transcription vectors (Stratagene) containing the full-length cDNA for the p55 and p75 murine TNF receptors were generously provided by Dr. Raymond Goodwin of Immunex Corporation [21]. Detailed protocols for probe synthesis, prehybridization, hybridization, post-hybridization, and autoradiographic localization have been published previously [14]. In brief, high specific activity sense and antisense riboprobes were synthesized by incorporation of both ³⁵S-labelled CTP and UTP with specific

activities greater than 800 Ci/mmol with the Riboprobe II System (Promega). Full-length templates used to generate antisense probes were obtained by linearization of the p55 and p75 cDNAs with Bgl II and Eco RI, respectively, and by use of T3 RNA polymerase for transcription. Full-length p55 and p75 sense probes used as controls were linearized with BamH I and Sac I, respectively, and transcribed with T7 RNA polymerase. Mounted sections were further postfixed in 10% neutral buffered formalin for 30 min, after which they were treated with proteinase K, acetylated, and dehydrated. The ³⁵S-labeled cRNA probes were diluted in hybridization buffer at 10⁷ cpm/ml, applied to the sections, and allowed to hybridize at 55–60°C for 12–24 h. Sections were then treated with RNase A to reduce background, and washed through progressively lower concentrations of saline-sodium citrate to reduce the salt content and increase the stringency of hybridization. Finally, sections were dehydrated, exposed to Beta Max film (Amersham) for 4–7 days, dipped in Kodak nuclear emulsion NTB3, dried, exposed for 2–4 weeks, developed, and counterstained with hematoxylin–eosin.

3. Results

3.1. Uninfected trigeminal ganglia

A total of 12 trigeminal ganglia from 8 animals were hybridized with antisense probes for mRNA encoding p55 and p75 TNFR subtypes. In each case, signal was observed over many, but not all, trigeminal sensory neurons in all portions of the ganglia, and diffusely over the arachnoid layers surrounding the trigeminal ganglia (Figs. 1–3). Signal for p55 TNFR mRNA was qualitatively denser than that for p75 TNFR mRNA in every case. While signal over some neuroglial cells could not be excluded with certainty, particularly over those cells in close approximation to intensely labeled trigeminal sensory neurons, signal over neuroglial cells in axon-rich portions of the trigeminal ganglia was comparable to background with both p55 and p75 riboprobes.

3.2. HSV-infected trigeminal ganglia

A total of eight trigeminal ganglia from four animals acutely infected with McKrae strain HSV 3 days previously were hybridized with antisense probes for mRNA encoding the p55 and p75 TNFR subtypes. Ganglia were taken from those animals previously shown to have an active and intense acute ocular infection [24]. In each case, signal for mRNA encoding both p55 and p75 was observed over trigeminal sensory neurons in all portions of the ganglia, and over the arachnoid layers surrounding the ganglia, with no qualitative difference in the distribution or intensity of mRNA signal as compared to uninfected trigeminal ganglia. However, ocular HSV infection was

accompanied by a marked leukocytic infiltrate into the medial, or ophthalmic, portion of the trigeminal ganglia, and an intense autoradiographic signal for both p55 and

p75 was observed over infiltrating white blood cells in this setting (Figs. 1 and 2). Again, while label over those neuroglial cells in close approximation to intensely labeled

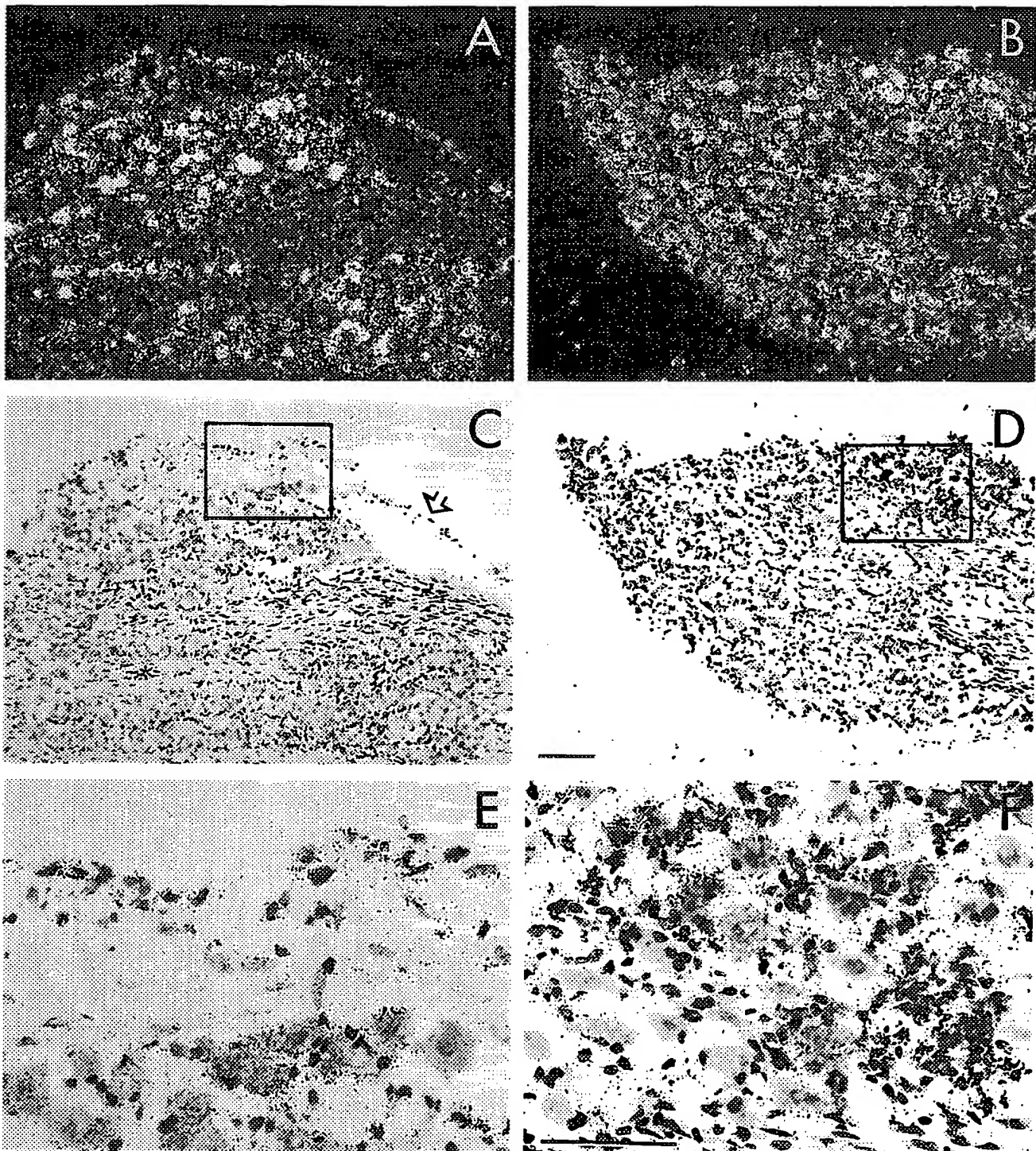


Fig. 1. Darkfield (A,B) and brightfield (C–F) photomicrographs of sections through normal (left panel) and HSV-infected (right panel) trigeminal ganglia demonstrating the in situ distribution of autoradiographic signal for mRNA encoding the p55 TNF receptor. Intense signal was observed over many, but not all, sensory neurons in all portions of both normal and HSV-infected ganglia (E,F). Although not addressed quantitatively, signal intensity appeared not to increase over trigeminal sensory neurons following infection (F). Heavy signal was also present over the surrounding arachnoid layers in both normal and HSV-infected ganglia (C, open arrow), and over infiltrating white blood cells in the medial portion of the trigeminal ganglia following ocular infection (right panel, see F). Axon-rich portions of the sections are labeled (*). Magnified photomicrographs (E,F) are taken from boxed areas of low-power brightfield images (C,D). Sections were counter-stained with hematoxylin–eosin. Scale bars at 200 μ m.

trigeminal sensory neurons could not be absolutely excluded, the signal over neuroglial cells in axon-rich portions of the ganglia was comparable to background for both p55 and p75.

3.3. Controls

A total of five uninfected and four infected trigeminal ganglia were hybridized with sense probes for mRNA

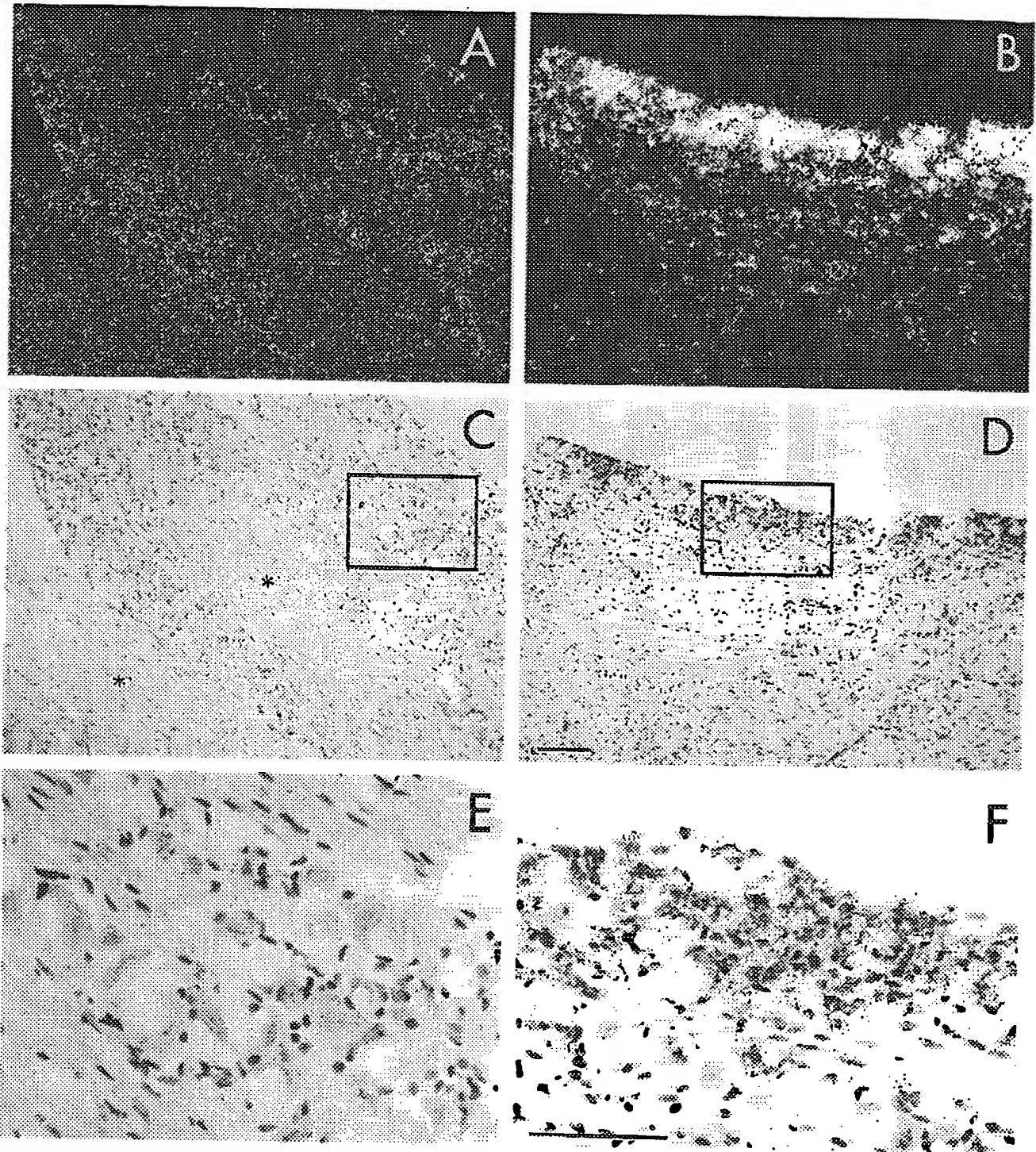


Fig. 2. Darkfield (A,B) and brightfield (C–F) photomicrographs of sections through normal (left panel) and HSV-infected (right panel) trigeminal ganglia demonstrating the in situ distribution of autoradiographic signal for mRNA encoding the p75 TNF receptor. Light signal was observed over many, but not all, sensory neurons in all portions of both normal and HSV-infected ganglia (E,F). Signal intensity appeared not to increase over trigeminal sensory neurons following infection, although intense signal was observed over infiltrating white blood cells in the medial, or ophthalmic, portion of the trigeminal ganglia in this setting (right panel, see F). Light signal was also present over the surrounding arachnoid layers in both normal and HSV-infected ganglia (not shown). Axon-rich portions of the sections are labeled (*). Magnified photomicrographs (E,F) are taken from boxed areas of low-power brightfield images (C,D). Sections were counter-stained with hematoxylin–eosin. Scale bars at 200 μ m.

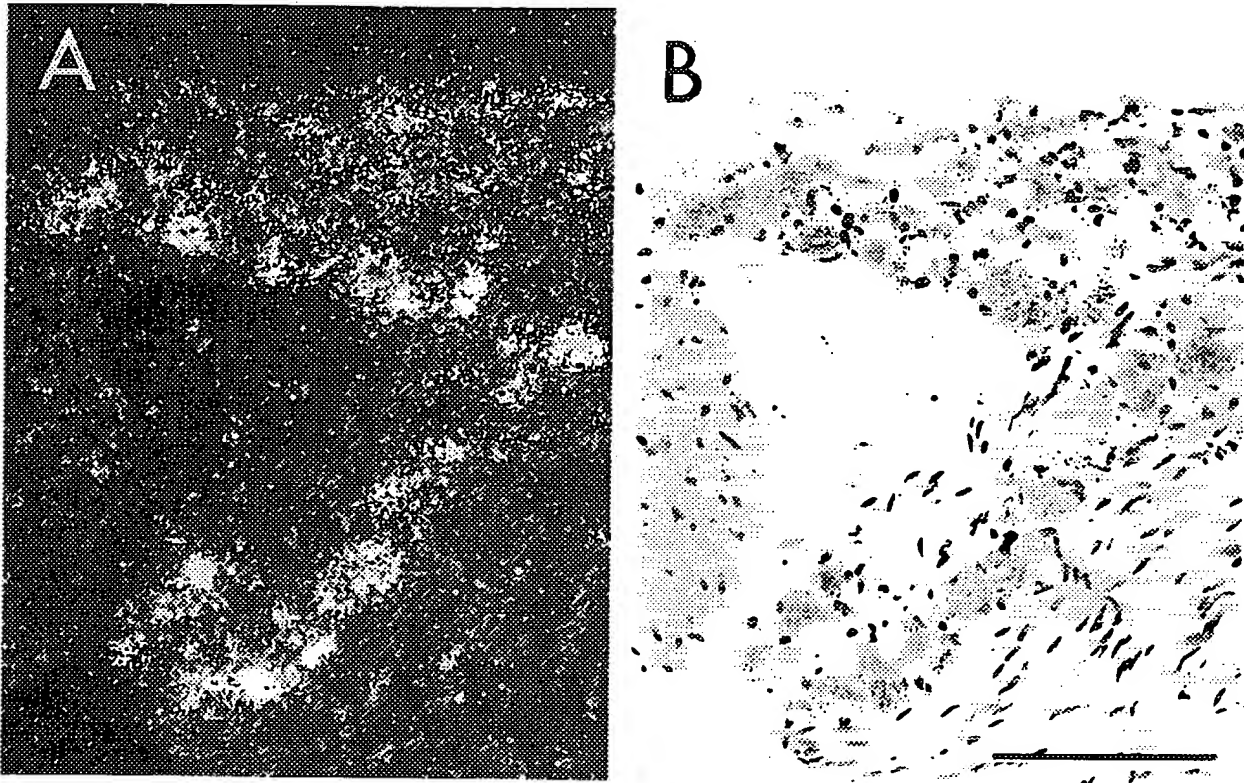


Fig. 3. High-power darkfield (A) and brightfield (B) photomicrographs of the same section through a normal trigeminal ganglion to show the in situ distribution of autoradiographic signal for mRNA encoding the p55 TNF receptor. Heavy signal is present over many, but not all neurons. Sections were counter-stained with hematoxylin–eosin. Scale bar at 200 μ m.

encoding the p55 and p75 the TNFR subtypes. In each case, autoradiographic signal over both the trigeminal ganglia and their surrounding arachnoid layers was compara-

ble to background (Fig. 4). In addition, we recently used a sensitive and specific RNA protection assay [54] to demonstrate mRNA encoding both TNFR subtypes in normal

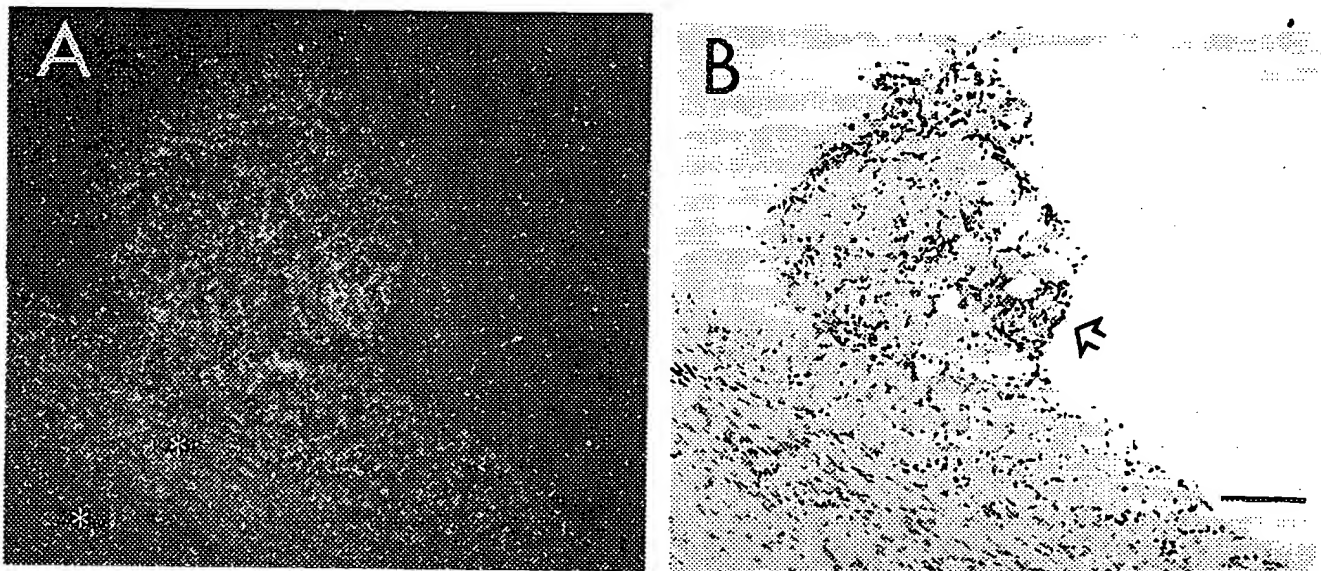


Fig. 4. Control darkfield (A) and brightfield (B) photomicrographs of the same section through the medial, or ophthalmic, portion of an HSV-infected trigeminal ganglion demonstrating the in situ distribution of autoradiographic signal using a sense probe for mRNA encoding the p55 TNF receptor. Signal using this, as well as the sense riboprobe for the p75 TNF receptor, was comparable to background. Axon-rich portions of the section are labeled (*). Sections were counter-stained with hematoxylin–eosin. Scale bar at 200 μ m.

murine trigeminal ganglia (unpublished observation), supporting our *in situ* results. Lastly, these TNFR probes have been used in both uninfected and HSV infected mouse eye [15] and lymphocytic choriomeningitis virus (LCMV)-infected brain [53,54] with high sensitivity and specificity.

4. Discussion

In situ hybridization was used to localize mRNA encoding the p55 and p75 TNFR subtypes in normal murine trigeminal ganglia, and in murine trigeminal ganglia acutely infected with McKrae strain HSV. Signal for mRNA encoding both TNFR subtypes was present over many, but not all, trigeminal sensory neurons in all portions of the ganglia, and over the arachnoid layers surrounding the trigeminal ganglia. Acute ocular infection with McKrae strain HSV appeared not to qualitatively affect the distribution or intensity of p55 or p75 mRNA signal over trigeminal sensory neurons or cells in the arachnoid layers, but was accompanied by a marked white blood cell infiltrate into the medial, or ophthalmic, portion of the trigeminal ganglia. In this setting, infiltrating white blood cells expressed high levels of mRNA for both TNFR subtypes.

Autoradiographic signal could not be identified over isolated trigeminal neuroglial cells in either normal or HSV-infected animals, a finding most evident over axon-rich portions of the ganglia. However, due to the relatively high energy of ^{35}S -labeled riboprobes, the close approximation of stellate neuroglial cells to trigeminal sensory neurons, and the loss of cellular morphology following the heavy proteinase K treatment required by *in situ* hybridization protocols [14], the possibility that some neuroglial cells in the immediate vicinity of TNFR expressing trigeminal neurons, specifically satellite cells, were also labeled by p55 and/or p75 riboprobes, cannot be excluded with certainty.

While this study represents the first demonstration of TNFRs on neurons in the peripheral nervous system, considerable anatomical evidence supports the existence of TNFR production by neurons and/or neuroglial cells in the central nervous system. Kinouchi and associates were the first to suggest the presence of high affinity TNFR sites in mouse brain homogenates using ^{125}I -murine-TNF [28]. Stalder and Campbell confirmed and extended this finding using a sensitive RNase protection assay, thus identifying mRNA encoding both the p55 and p75 TNFR subtypes in whole mouse brain RNA preparations [54]. More recently, *in vitro* studies have demonstrated mRNA [25] and immunoreactivity [13] for the p55 TNFR subtype in rat hippocampal and cortical neurons, as well as high affinity binding of ^{125}I -murine-TNF to mouse astrocytes via a 58 kDa receptor, presumed to be p55 [3,6].

The cellular function of neuronal and neuroglial TNFRs appears complex, with several studies suggesting a role for TNF in the regulation of both neurotransmission and cell

survival. In the central nervous system, TNF has been shown to alter synaptic activity in rat hippocampal slice [56] and culture [25], to regulate the release of norepinephrine from nerve terminals in the rat hippocampus [26] and median eminence [16], and, paradoxically, to both potentiate [12,20,55], and abrogate [18] the toxic effects of such stimuli as glucose deprivation and excitatory amino acid exposure. The effect of TNF on central neuroglial cells has been less well studied, although TNF has been shown to decrease potassium currents [50] and promote apoptosis [32,41] in oligodendrocytes. In astrocytes, TNF has been shown to increase C3 gene expression [7], cause proliferation [46], and enhance the interferon- γ mediated expression of class II antigens [8], a phenomenon of considerable potential relevance given the proposed role of neuroglial class II antigens in limiting HSV infection in the central nervous system [30]. In the periphery, TNF has been shown to enhance neurotransmitter release from motoneuron endplates [10], and to affect both calcium currents [49] and nicotinic responses [51] in cultured sympathetic neurons. In addition, TNF has been shown in cultured Schwann cells to inhibit proliferation, to decrease gap junction conduction [11], and to down-regulate myelin-associated glycoprotein expression, thereby inhibiting neurite outgrowth [45]. However, we found no evidence for expression of TNFR mRNA over Schwann cells in either normal or infected trigeminal ganglia, suggesting that TNFR mRNA is expressed below the level of detection with radiolabeled riboprobes, or, alternatively, that those TNF-mediated Schwann cell responses observed *in vitro* are either indirect, or related to cell growth in culture.

Autoradiographic signal for both TNFR subtypes was observed over the arachnoid layers surrounding the trigeminal ganglia. This is an important finding given the well documented increase in TNF in the central nervous system in the setting of meningitis [2,4,22,31,35–38,40,60], where cerebral spinal fluid levels of TNF have been inversely correlated with level of consciousness [2,4,35,36], and local TNF has been shown to elicit many of the injurious cerebral metabolic and circulatory changes that accompany this condition [35,44,58]. Recently, Sanna and associates have shown that intracerebroventricular administration of the bacterial lipopolysaccharide, a component of the outer membrane of Gram-negative bacteria, mimics the rise in central TNF seen with bacterial meningitis [43].

Acute ocular HSV infection was accompanied by an intense leukocytic infiltrate into the medial, or ophthalmic, portion of the trigeminal ganglion. Our *in situ* hybridization results demonstrated that these infiltrating white blood cells expressed high levels of both p55 and p75 TNFR mRNA. While a slight increase in or delayed expression of endogenous TNFR mRNA cannot be ruled out with these studies, the results strongly suggest that the increase in TNFR mRNA in the trigeminal ganglia observed in the setting of acute ocular HSV infection is due to an influx of receptor-bearing white blood cells. Similar findings have

been reported for TNFR mRNA in mouse brain following infection with LCMV [53], and in eye after acute infection with McKrae strain HSV [15].

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